COMPOUNDS AND METHODS FOR INHIBITION OF PHOSPHOLIPASE A2 AND CYCLOOXYGENASE-2

REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Application Ser. No. 60/278,257 filed Mar. 23, 2001.

FIELD OF THE INVENTION

The present invention relates to a group of compounds and a method for inhibiting certain enzyme systems that mediate a variety of physiological responses in mammals. More particularly, the present invention relates to a compound and method for inhibition of phospholipase A_2, and cyclooxygenase-2, which are enzymes that catalyze a cascade of biochemical reactions that lead to the mediation of pain, fever, inflammation and other functions.

BACKGROUND OF THE INVENTION

Human beings and other mammals spend energy continuously defending against a vast array of invasive pathogenic organisms including bacteria, viruses, fungi and other intracellular and extra-cellular parasites in addition to other potentially harmful agents that are capable of upsetting homeostasis. In response, humans and other mammals maintain many mechanisms capable of processing and defending against such antigens and agents. The biological response to attack and injury is mediated through the formation of a series of structurally related compounds called eicosanoids, which include the prostaglandins, the leukotrienes, and the thromboxanes. Master enzymes known as phospholipase A_2 and phospholipase C regulate the formation of these highly potent compounds.

Phospholipase A_2 is a heat-stable, calcium dependent enzyme that catalyses the hydrolysis of the 2-acyl bond of 3-n-phosphoglycerides. It has a molecular weight of about 30,000 Daltons. Phospholipase A_2 has been found in many human tissues including platelets, chondrocytes, placenta, cartilage, peritoneal calls and peritoneal fluid and spleen. (Vades, p., Puzanski, W., Soluble phospholipase A_2 in human pathology: clinical-laboratory interface. Biochemistry, molecular biology, and physiology of phospholipase A_2 and its regulatory factors. Ed AB Mukherjee, Plenum Press, New York, 1990.) High levels of phospholipase A_2 are found in synovial tissue and it has been shown that activity of rheumatoid arthritis significantly correlated to the levels of serum phospholipase A_2. (Vadas, P., Pruzanski, W. and Stefanski, E., Characterization of extracellular phospholipaseA2 in human synovial fluids. Life Sci. 36: 579, 1985.)

Substantial evidence has been found (above reference) that excessive concentrations of extra-cellular phospholipase A_2 may initiate and propagate inflammation and cause cellular damage. In addition phospholipase A_2 was also found to modulate various aspects of phagocytic activity, vascular tone and permeability. A strong correlation between phospholipase A_2 activity and certain human diseases have also been identified. A few such diseases are listed in table 1 below.

DISEASE	LOCATION OF PHOSPHOLIPASE A_2
Rheumatoid arthritis	Serum, synovial fluid
Osteoarthritis	Synovial fluid
Psoriasis	Synovial fluid
Monoarthritis	Synovial fluid
Gout	Synovial fluid
Collagen Vascular Disease	Serum
Pancreatitis	Serum
Peritonitis	Peritoneal fluid
Sepsis and Shock	Serum
Renal Failure	Serum

FIG. 1 illustrates the key role that phospholipase A_2 and cyclooxygenase-2 are currently understood to play in the formation of potent, biologically active substances that mediate a variety of conditions and disease states. The process of the formation of prostaglandin's, leukotrienes, lipoxins, and thromboxanes in addition to lysosomal enzyme release, bactericidal activity, pro-inflammatory eicosanoids, PAF and lysophosphatides and reactive oxygen species, begins on the surface of specialized cells including osteoblasts, endothelial cells, chondrocytes, synoviocytes, and renal mesangial cells. The major constituents of these, and all other, cell membranes are phospholipids. The biochemical conversion of these important molecules to arachidonic acid is catalyzed by phospholipase A_2. Arachidonic acid is further converted to leukotrienes, lipoxins, thromboxanes and prostaglandins. The latter two species are formed by way of chemical conversion catalyzed by two other important enzymes known as cyclo-oxygnease-1 and cyclooxygenase-2. The arachidonic acid cascade is a well know pathway leading to the mediation of pyrogenicity, vasoconstriction, increased vascular permeability, contraction of smooth muscle, inflammation, and pain.

Found in every cell of the human body, albeit to varying degrees, prostaglandins have profound physiologic effects including mediation of pain and inflammation. The prostaglandins (PG) are a family of lipid-soluble hormone-like molecules produced by different cell types in the body. For example, macrophages and monocytes are large producers of both PGE2 and PGF2, neutrophils produce moderate amounts of PGE2, and masts cells produce PGD2. It is important to note that, unlike histamine, prostaglandins do not exist free in tissues vacuoles, but have to be synthesized and released in response to an appropriate stimulus. This synthesis is dependent on phospholipase A_2 and cyclooxygenase-2.

Thromboxanes are produced by monocytes and macrophages, as well as by platelets. Thromboxanes are involved in causing platelets to aggregate and constrict blood vessels and airways. These effects are some what opposed by the action of prostacyclin (PGI2), which is a potent vasodilator.

Leukotrienes (LT) exist in a number of varieties, and cause the chemotaxis (directed locomotion) and/or chemokinesis (general cell movement) of a number of cell types including neutrophils. The synthesis of LTB4 is inhibited by colchicines, an anti-flammatory agent used for treatment of gout. The mixture of LTC4, LTD4 and LTE4 originally called slow reacting substance of anaphylaxis is produced by a wide variety of smooth muscle, mainly in the bronchus, and have effects on mucous secretions. Inhibition of the formation of these substances is a useful therapeutic modality in asthma.

Lipoxins (LX) are a family of molecules that are thought to stimulate changes in microcirculation. For example,